

IN THE CLAIMS

Please amend the claims as follows:

Claim 1 (Previously Presented): An isolated polynucleotide which encodes a thermostable polypeptide having DNA polymerase activity comprising:

an amino acid sequence having at least 95% identity to SEQ ID NO: 26,

wherein said polypeptide has a mutation at residue 484 of SEQ ID NO: 26 which replaces the methionine residue (Met) with a different amino acid residue.

Claim 2 (Previously Presented): The isolated polynucleotide of Claim 1, which encodes a polypeptide having one of the following mutations: M484V or M484T.

Claim 3 (Previously Presented): The isolated polynucleotide of Claim 1, wherein said polypeptide further comprises at least one mutation selected from the group consisting of A331T, S335N, M470K, M470R, F472Y, and W550R.

Claim 4 (Previously Presented): The isolated polynucleotide of Claim 1, wherein said polypeptide further comprises at least one other mutation in amino acids 461 to 490 of SEQ ID NO: 26, or at a position selected from the group consisting of A331, L332, D333, Y334, S335, M470, F472, W550, L332, D333, and Y334.

Claim 5 (Previously Presented): The isolated polynucleotide of Claim 1, wherein said polypeptide has at least 97.5% identity to SEQ ID NO: 26.

Claim 6 (Previously Presented): The isolated polynucleotide of Claim 1, wherein said polypeptide comprises at least two mutations of the amino acid sequence of SEQ ID NO: 26.

Claim 7 (Previously Presented): The isolated polynucleotide of Claim 1, which comprises SEQ ID NO: 19.

Claim 8 (Previously Presented): The isolated polynucleotide of Claim 1, wherein said polypeptide comprises SEQ ID NO: 20.

Claim 9 (Withdrawn): The isolated polynucleotide of Claim 1, which comprises SEQ ID NO: 37.

Claim 10 (Withdrawn): The isolated polynucleotide of Claim 1, which encodes a polypeptide comprising SEQ ID NO: 38.

Claims 11-16 (Cancelled)

Claim 17 (Previously Presented): The isolated polynucleotide of Claim 1, consisting of SEQ ID NO: 19, or SEQ ID NO: 37.

Claim 18 (Previously Presented): An isolated polynucleotide that is fully complementary to the polynucleotide of Claim 1.

Claim 19 (Previously Presented): An isolated polynucleotide which hybridizes under stringent conditions to the polynucleotide of Claim 1; and which encodes a polypeptide that has a mutation at residue 484 of SEQ ID NO: 26 which replaces the methionine residue (Met) with a different amino acid residue;

wherein said stringent conditions comprise washing in 0.1X SSC at a temperature of 68°C.

Claim 20 (Previously Presented): A vector comprising the isolated polynucleotide of Claim 1.

Claim 21 (Original): The vector of Claim 20, wherein said polynucleotide is operably linked to a heterologous expression sequence.

Claim 22 (Previously Presented): An isolated host cell comprising the isolated polynucleotide of Claim 1.

Claim 23 (Withdrawn): An isolated thermostable polypeptide comprising an amino acid sequence having at least 95% identity to SEQ ID NO: 26, wherein said polypeptide has at least one mutation in amino acids 461 to 490 of SEQ ID NO: 26, or at a position selected from the group consisting of A331, L332, D333, Y334, S335, M470, F472, M484, W550, L332, D333, and Y334, and wherein said polypeptide has DNA polymerase activity.

Claim 24 (Withdrawn): The isolated polypeptide of Claim 22, wherein said at least one mutation is selected from the group consisting of A331T, S335N, M470K, M470R, F472Y, M484V, M484T, and W550R.

Claim 25 (Withdrawn): The purified polypeptide of Claim 23, wherein said polypeptide has at least 90% identity to SEQ ID NO: 26.

Claim 26 (Withdrawn): The isolated polypeptide of Claim 23, wherein said polypeptide has at least 95% identity to SEQ ID NO: 26.

Claim 27 (Withdrawn): The isolated polypeptide of Claim 23, wherein said polypeptide has at least 97.5% identity to SEQ ID NO: 26.

Claim 28 (Withdrawn): The isolated polypeptide of Claim 23, wherein said polypeptide wherein said polypeptide comprises at least two mutations.

Claim 29 (Withdrawn): The isolated polypeptide of Claim 23, wherein said polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, and SEQ ID NO: 38.

Claim 30 (Withdrawn): The isolated polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 20.

Claim 31 (Withdrawn): The isolated polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 22.

Claim 32 (Withdrawn): The isolated polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 24.

Claim 33 (Withdrawn): The isolated polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 28.

Claim 34 (Withdrawn): The isolated polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 30.

Claim 35 (Withdrawn): The isolated polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 32.

Claim 36 (Withdrawn): The isolated polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 34.

Claim 37 (Withdrawn): The isolated polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 36.

Claim 38 (Withdrawn): The isolated polypeptide of Claim 29, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 38.

Claim 39 (Withdrawn): A kit for amplifying DNA comprising:  
an isolated thermostable polypeptide, wherein said polypeptide has at least 95% homology to SEQ ID NO: 26, wherein said polypeptide has at least one mutation in amino acids 461 to 490 of SEQ ID NO: 26, or at a position selected from the group consisting of A331, L332, D333, Y334, S335, M470, F472, M484, W550, L332, D333, and Y334, and wherein said polypeptide has DNA polymerase activity;  
a concentrated buffer solution, and optionally one or more divalent metal ions; and  
a mixture of deoxyribonucleotides.

Claim 40 (Withdrawn): The kit of Claim 39, wherein said at least one mutation is selected from the group consisting of A331T, S335N, M470K, M470R, F472Y, M484V, M484T, and W550R.

Claim 41 (Withdrawn): The kit of Claim 39, wherein said divalent metal ion is  $Mg^{2+}$  or  $Mn^{2+}$ .

Claim 42 (Withdrawn): The kit of Claim 39, wherein said polypeptide has at least 90% identity to SEQ ID NO: 26.

Claim 43 (Withdrawn): The kit of Claim 39, wherein said polypeptide has at least 95% identity to SEQ ID NO: 26.

Claim 44 (Withdrawn): The kit of Claim 39, wherein said polypeptide has at least 97.5% identity to SEQ ID NO: 26.

Claim 45 (Withdrawn): The kit of Claim 39, wherein said polypeptide comprises at least two mutations.

Claim 46 (Withdrawn): The kit of Claim 39, wherein said polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, and SEQ ID NO: 38.

Claim 47 (Withdrawn): The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 20.

Claim 48 (Withdrawn): The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 22.

Claim 49 (Withdrawn): The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 24.

Claim 50 (Withdrawn): The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 28.

Claim 51 (Withdrawn): The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 30.

Claim 52 (Withdrawn): The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 32.

Claim 53 (Withdrawn): The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 34.

Claim 54 (Withdrawn): The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 36.

Claim 55 (Withdrawn): The kit of Claim 46, wherein said polypeptide has the amino acid sequence of SEQ ID NO: 38.

Claim 56 (Withdrawn): The kit of Claim 39, further comprising a 5' to 3' exonuclease or a 3' to 5' exonuclease.

Claim 57 (Withdrawn): The kit of Claim 56, wherein said 5' to 3' exonuclease has SEQ ID NO: 50.

Claim 58 (Withdrawn): The kit of Claim 56, wherein said 3' to 5' exonuclease has SEQ ID NO: 51.

Claim 59 (Withdrawn): A method for reverse transcribing an RNA comprising:

a) providing a reverse transcription reaction mixture comprising said RNA, a primer, a divalent cation, and a purified thermostable polypeptide comprising an amino acid sequence having at least 80% identity to SEQ ID NO: 26, wherein said polypeptide has at least one mutation in amino acids 461-490 of SEQ ID NO: 26, or at a position selected from the group consisting of A331, L332, D333, Y334, S335, M470, F472, M484, W550, L332, D333, and Y334, and wherein said polypeptide has DNA polymerase activity.; and

b) treating said reaction mixture at a temperature and under conditions suitable for said purified polypeptide to initiate synthesis of an extension product of said primer to provide a cDNA molecule complementary to said RNA.



Claim 60 (Withdrawn): The method of Claim 59, wherein said at least one mutation is selected from the group consisting of A331T, S335N, M470K, M470R, F472Y, M484V, M484T, and W550R.

Claim 61 (Withdrawn): A method of identifying thermostable mutant polypeptides comprising

a) packaging a vector in which a polynucleotide encoding a phage coat protein is fused to a polynucleotide encoding a protein having at least 80% identity to SEQ ID NO: 26 into a phage;

- b) expressing the fusion protein;
- c) isolation of phage particles;
- d) infecting E. coli and incubating the infected E. coli;
- e) detecting the fusion protein;
- f) assessing polymerase activity.

Claim 62 (Withdrawn): The method of Claim 61, wherein (b) – (f) are repeated 0 to 25 times.

Claim 63 (Withdrawn): The method of Claim 61, wherein the phage coat protein is SEQ ID NO: 39.

Claim 64 (Withdrawn): A method of identifying thermostable mutant polypeptides having a catalytic activity comprising:

a) packaging a vector in which a gene or fragment thereof encoding variants of a catalytic domain responsible for the catalytic activity fused to a gene encoding a phage coat protein;

b) isolation and purification of phage particles;

c) heating the phage-mutant polypeptide at a temperature ranging from 50°C to 90°C for a time ranging from 30 seconds to several hours;

d) cross-linking a specific substrate with a phage particle;

e) forming a reaction product from the substrate catalyzed by the thermostable mutant protein on phage, wherein the temperature is optionally regulated to be the same or greater or lower than the temperature of (c)

f) selecting the phage particles comprising a variant nucleotidic sequence encoding for the catalytic domain responsible for the catalytic activity at the regulated temperature, by capturing the reaction product or screening for said reaction product,

g) infecting *E. coli* with the phage particles selected at step (f),

h) incubating the infected *E. coli*; and

i) assessing catalytic activity of the proteins corresponding to isolated genes.

Claim 65 (Withdrawn): The method of Claim 64, wherein the gene or fragment thereof encoding variants of a catalytic domain is directly fused to the gene encoding a phage coat protein.

Claim 66 (Withdrawn): The method of Claim 64, wherein the steps (a) to (h) are repeated 0 to 20 times.

Claim 67 (Withdrawn): The method of Claim 64, wherein the gene or fragment thereof encoding variants of a catalytic domain and the gene encoding a phage coat protein, are indirectly fused by a peptide or polypeptide linker.

Claim 68 (Withdrawn): The method of Claim 67, wherein the peptide is selected from the group consisting of:

a glycine rich linker such as (SG<sub>4</sub>)<sub>n</sub> (SEQ ID NO: 39),

a human calmodulin (SEQ ID NO: 46), and

a hexahistidine binding single chain variable fragment consisting of:

(i) an anti-His Tag Antibody 3D5 Variable Heavy Chain (SEQ ID NO: 47),

(ii) a linker (SEQ ID NO: 48),

(iii) an anti-His Tag Antibody 3D5 Variable Light Chain (SEQ ID NO: 49).

Claim 69 (Withdrawn): The method of Claim 67, wherein the polypeptide linker is selected from the group consisting of:

- a protein binding the substrate at high temperature

- a catalytic domain of a 5' to 3' exonuclease

- a catalytic domain of a 3' to 5'

- a catalytic domain of *Bacillus circulans* cyclodextringlycosyltransferase (SEQ ID NO: 52),

- a catalytic domain of *Bordetella pertussis* adenylate cyclase (SEQ ID NO: 53)

- a *Bacillus amyloliquefaciens* serine protease subtilisin (SEQ ID NO: 54), and

- a catalytic domain of *Bacillus subtilis* lipase A (SEQ ID NO: 55).

Claim 70 (Withdrawn): The method of Claim 64, wherein the cross-linking between the specific substrate of the catalytic domain of the polypeptide with the phage particle is made by a cross-linking agent selected from the group consisting of a:

- maleimidyl group
- iodoacetyl group
- disulfide derivative and
- any other thermostable link.

Claim 71 (Withdrawn): The method of Claim 64, wherein the catalytic domain is the catalytic domain of an enzyme selected from the group consisting of a:

- DNA polymerase,
- alpha-amylase,
- lipase,
- protease,
- a cyclodextringlycosyltransferase, and
- an adenylate cyclase.

Claim 72 (Withdrawn): The method of Claim 64, wherein the assessment of the catalytic activity of (f) is made by means of a DNA polymerization.

Claim 73 (Withdrawn): The method of Claim 64, wherein (b) is performed after (e) or during (h).

Claim 74 (Withdrawn): The method of Claim 64, wherein the temperature in (e) is regulated to be the same or greater than the temperature of (c).

Claim 75 (Withdrawn): The method of Claim 64, wherein the temperature in (e) is regulated to be the same or less than the temperature of (c).

Claim 76 (Withdrawn): A method of obtaining a thermostable variant enzyme comprising:

- a) screening enzymes expressed at the surface of phage particles and identifying at least a thermostable variant conserving its active; catalytic domain at regulated temperature according to the method of claim 61,
- b) isolating and sequencing a DNA encoding said identified thermostable variant;
- c) preparing a vector comprising the DNA of step (b);
- d) transfecting or infecting cells with the vector obtained at step c);
- e) expressing the thermostable variant enzyme from the cells and optionally,
- f) recovering, isolating and purifying said thermostable variant enzyme expressed at step (e).

Claim 77 (Withdrawn): A method of obtaining a thermostable variant enzyme comprising:

- a) screening enzymes expressed at the surface of phage particles and identifying at least a thermostable variant conserving its active; catalytic domain at regulated temperature according to the method of claim 69,
- b) isolating and sequencing a DNA encoding said identified thermostable variant;

- c) preparing a vector comprising the DNA of step (b);
- d) transfecting or infecting cells with the vector obtained at step c);
- e) expressing the thermostable variant enzyme from the cells and optionally,
- f) recovering, isolating and purifying said thermostable variant enzyme expressed at step (e).

Claim 78 (Previously Presented): A monoclonal phage selected from the group consisting of SJL q (CNCM I-3168), SJL d (CNCM I-3169), SJL I (CNCM I-3170), SJL s (CNCM I-3171), SJL b (CNCM I-3172), SJL n (CNCM I-3173), SJL g (CNCM I-3174), SJL m (CNCM I-3175), and SJL a (CNCM I-3176).

Claim 79 (Previously Presented): An isolated recombinant host cell comprising the phage of claim 78.

Claim 80 (New): The isolated polynucleotide of claim 1 which encodes a polypeptide having DNA polymerase activity and reverse transcriptase activity in the absence of manganese.